Transmission of respiratory syncytial virus at the paediatric intensive-care unit: a prospective study using real-time PCR

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Abstract

Transmission of respiratory syncytial virus (RSV) from children with lower respiratory tract infection (LRTI) at a paediatric intensive-care unit (PICU) was examined using a highly sensitive real-time PCR. Twenty-four children with RSV LRTI were admitted during the study period (total days of potential transmission: 239). Forty-eight RSV-negative patients were followed up for RSV acquisition every 5 days (total days of exposure: 683). No single RSV transmission was documented with this highly sensitive diagnostic method. Therefore, routine infection control measures of LRTI patients seem to be adequate to prevent RSV transmission at the PICU.

Keywords: Nosocomial infections, paediatric intensive-care units, PCR, prospective follow-up study, respiratory syncytial virus, respiratory tract infections

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Community-acquired respiratory syncytial virus (RSV) lower respiratory tract infection (LRTI) is a common reason for admission to a paediatric intensive-care unit (PICU) [1–3]. RSV has been shown to be transmitted to other patients, causing nosocomial infections with significant morbidity [4]. Research on the frequency of transmission of RSV at the PICU has shown contradictory results [5–8]. RSV detection has traditionally been performed with direct immunofluorescence (DIF) and viral culture. Real-time PCR is a more sensitive detection method than DIF and viral culture [9], but is also more expensive. This is the first study using real-time PCR to prospectively reveal RSV transmission from children with RSV LRTI to other children admitted to a PICU. To confirm that RSV acquisition was nosocomial, patients were assessed for asymptomatic carriage of RSV upon admission to the PICU, and subsequently every 5 days.

During the RSV season of 2005–2006, 24 patients with RSV LRTI were admitted to the PICU of Wilhelmina Children's Hospital. Every 5 days, it was determined whether patients were still shedding virus. If a patient with RSV LRTI was admitted to the unit, other patients without LRTI who were present in the unit or admitted to the unit were tested for RSV. This surveillance was continued during all periods in which patients were shedding RSV ('RSV-positive periods'). Exclusion criteria were as follows: (i) post-head trauma status; (ii) discomfort of sampling was deemed to be unethical; and (iii) PICU stay <12 h/weekend only. As RSV surveillance of non-LRTI patients was part of routine infection control measures; according to the Medical Ethical Research Council of the institution, there was no need for patient consent/ethical approval. Every child without LRTI who stayed at the PICU for >3 days was eligible for follow-up. For logistic reasons, all children at the unit were sampled on the same day ('sample day'), every 5 days.

RSV detection was performed by examining nasopharyngeal aspirates in intubated children and throat swabs in extubated children. Specimens were examined with DIF, viral culture and real-time PCR as previously described [9–11]. In short, part of the sample was inoculated onto HEp cells for viral culture, and examined twice weekly for cytopathogenic effect. A second aliquot was used for DIF detection (Imagen; DaKo, Glostrup, Denmark). DIF was not performed on throat swabs. The last part of the sample was used for real-time PCR [9–11]. Briefly, after nucleic acid extraction (MagNA pure LC; Roche Diagnostics, Basel, Switzerland), cDNA was synthesized using MultiScribe reverse transcriptase and random hexamers (both from Applied Biosystems, Foster City, CA, USA). Finally, amplification was carried out in duplicate in a 25-μL reaction mixture. Murine encephalomyocarditis virus was used as an internal control virus to

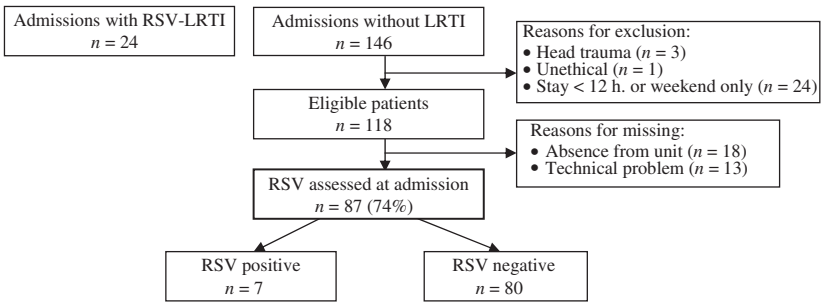

FIG. 1. Flow of patients included in the study during the respiratory syncytial virus (RSV)-positive periods. LRTI, lower respiratory tract infection.

monitor for efficient extraction, cDNA synthesis, and amplification [12]. As transmission of RSV occurs mainly through contact with objects rather than through aerosols, routine infection control measures for LRTI patients included rigorous hand hygiene, together with gloving and gowning.

During the study period, 24 RSV LRTI patients were admitted, staying for a mean of 10 days (standard deviation: 4.4 days). Follow-up samples remained positive for a mean of 7 days (standard deviation: 4.8 days). In total, RSV LRTI patients were shedding virus for 239 days. During the RSV-positive periods, 87 children were tested for RSV upon admission (Fig. 1; 58 nasopharyngeal aspirates and 29 throat swabs).

Seven of the 87 examined patients were found to be asymptomatic carriers of RSV (four identified by PCR and DIF, and three identified by PCR only). Age and length of stay were diverse among these patients. Most children had a significant medical history, and were admitted for surgical reasons. Notably, two children had suffered from an RSV infection 1 week prior to admission to the PICU. However, none of these patients showed any sign of LRTI during their entire stay at the PICU.

Fifty-eight patients stayed at the PICU for >3 days. Forty-eight patients (83%) stayed long enough to be present during at least one 'sample day', and follow-up results were therefore available. In total, non-LRTI patients were exposed for 683 days to RSV-shedding LRTI patients. None of the patients was found to acquire RSV during follow-up, regardless of whether real-time PCR, viral culture or DIF tests were analysed.

For the detection of RSV, real-time PCR was the viral test with the highest positivity rate. With real-time PCR, 31 RSV-positive patients were detected, whereas viral culture and DIF revealed 13 and 21 RSV-positive patients, respectively.

Several limitations of our study warrant further discussion. First, it was not possible to sample all eligible PICU patients to determine asymptomatic carriership, owing to early discharge, absence from the unit for additional tests or surgery, or technical problems. Second, owing to the study design, in which the entire PICU population was sampled on the same day ('sample day'), every 5 days, there was no follow-up sample for some patients who stayed for >3 days at the PICU. Third, RSV transmission was assessed only when patients with RSV LRTI were admitted to the PICU.

An unexpected finding of the study was that seven patients without LRTI were found to carry RSV upon admission. These asymptomatic RSV patients can shed RSV and cause nosocomial infections, especially because they are not recognized and no infection control measures are taken. RSV is believed to be shed for up to 2 weeks after symptoms occur; however, most of the literature on RSV shedding is based on viral culture results and not on more sensitive tests such as real-time PCR [13]. Thus, the length of time for which RSV patients shed RSV is not obvious. Therefore, the impact of asymptomatic shedders at the PICU must be the focus of further study, and these studies must maintain RSV surveillance during periods in which the PICU seems to be 'RSV-free'.

In conclusion, children with RSV LRTI admitted to the PICU during the RSV season were not found to transmit RSV to patients without LRTI, even though a highly sensitive real-time PCR assay was used. Therefore, routine infection control measures for LRTI patients seem to be adequate to prevent RSV transmission at the PICU.

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Transparency Declaration

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declare that they have no conflicting of interests in relation to this work.

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Detection of, and frequent co-infection with, human bocavirus in faecal specimens from children in Wuhan, China

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Abstract

A novel parvovirus, human bocavirus (HBoV), was first discovered in children with respiratory tract infections in Sweden. A retrospective study of HBoV in faecal samples from children suffering from diarrhea, covering a 3-year period (November 2000 to October 2003) in Wuhan, China, was undertaken. PCR assays were used to evaluate 214 faecal samples and to determine the role of HBoV in diarrhoea. Among 196 virus-infected children with diarrhoea, 2.55% were HBoV-positive; however, all HBoV-positive patients were co-infected with common enteric viruses. This result does not support the notion that HBoV is a viral agent causing acute diarrhoea.

Keywords: HBoV, children, faecal samples, PCR detection, co-infection

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In 2005, Allander *et al.*[1]described a previously uncharacterized virus in nasopharyngeal aspirates from children suffering from respiratory tract diseases, and provisionally named it human bocavirus (HBoV). Its global prevalence in respiratory samples from children, infants and rarely, adults with various respiratory diseases has been reported to be 3–19% [1–10]. Several studies have reported detection of HBoV in 0.8–9.1% of faecal samples from children with diarrhoea [8,11–13]. This newly identified virus has therefore been described as an enteric pathogen, and also a respiratory pathogen [3,4,10,14–16]. To elucidate the possible clinical role of HBoV infection, we retrospectively investigated faecal samples from children with diarrhoea in Wuhan, China, for the presence of the virus using PCR techniques. In this pilot